Functions and Regulatory Mechanisms of Sodium and Chloride Ion Transporters in Fish: Postprint

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Abstract

Sodium ions (Na+) and chloride ions (Cl-) not only regulate osmotic pressure balance in fish body fluids and participate in the maintenance of cell membrane resting potential, but are also indispensable for electrolyte homeostasis within the fish organism. Carrier proteins located on the epithelial cell membranes of the gills, gastrointestinal tract, and renal tubules of teleost fish—including Na+/potassium ion (K+)-ATPase, Na+-K+-2Cl- cotransporter, Na+/hydrogen ion (H+) exchanger, and cystic fibrosis transmembrane conductance regulator—constitute the primary regulatory pathways for Na+ and Cl- metabolism, and the expression of these transport proteins directly affects electrolyte balance within the organism. This review summarizes the functions of major carrier proteins associated with Na+ and Cl- transport in fish, factors influencing their activity, and their regulatory mechanisms.

Full Text

Function and Regulatory Mechanism of Na+ and Cl- Transporters in Fish

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Abstract: Sodium (Na+) and chloride (Cl-) ions not only participate in the regulation of osmotic pressure balance and resting membrane potential in fish, but are also essential for maintaining electrolyte homeostasis. Key transporter proteins located on the epithelial cell membranes of gills, gastrointestinal tract,

and renal tubules in teleosts—including Na+/K+-ATPase, Na+-K+-2Cl- cotransporter, Na+/H+ exchanger, and cystic fibrosis transmembrane conductance regulator—constitute the primary regulatory pathways for Na+ and Cl- metabolism. The expression of these transport proteins directly influences electrolyte balance. This review summarizes the functions, factors affecting their activity, and regulatory mechanisms of the major transporters involved in Na+ and Cl- transport in fish.

Keywords: fish; Na+; Cl-; transporter; osmoregulation

In most cases, teleost fish body fluids are in a non-isosmotic state relative to the environment, necessitating highly efficient ion-osmoregulatory mechanisms to maintain internal homeostasis and ensure normal biochemical and physiological processes. In freshwater environments, where fish body fluid osmolality exceeds that of the ambient water, fish must counteract mineral loss. Conversely, in marine environments where body fluid osmolality is lower, fish must prevent cellular dehydration caused by excessive salt influx. Euryhaline teleosts exhibit superior adaptability to environmental salinity changes due to their enhanced capacity for osmotic pressure regulation. While gills and kidneys are recognized as the primary osmoregulatory organs, recent studies have revealed that the gastrointestinal tract, as the main site for exogenous nutrient absorption, also participates in osmotic regulation by acquiring electrolytes from food and water.

Multiple ions in fish plasma contribute to osmotic balance regulation, including Na+, K+, Ca2+, Mg2+, Fe2+, Cu2+, Mn2+, Zn2+, and Cl-. Among these, Na+ and Cl- concentrations are the highest (Na+: 165–285 meq/L; Cl-: 129–270 meq/L) and thus play dominant roles in osmotic pressure regulation. Fish can absorb Na+ and Cl- through gills while also secreting excess ions from the blood. Research on rainbow trout (*Oncorhynchus mykiss*) demonstrated that dietary Na+ and K+ (approximately 90%) are primarily absorbed in the stomach, whereas in the agastric killifish (*Fundulus heteroclitus*), the intestine serves as the main site for Na+ absorption. Intestinal perfusion studies confirmed that killifish in freshwater exhibit higher Cl- absorption rates than those in seawater. Additionally, fish regulate mineral balance by modulating renal Na+ and Cl- reabsorption. In marine environments, Na+ secretion via gills exceeds that via kidneys, while in freshwater, branchial Na+ efflux is suppressed and kidneys compensate by reabsorbing Na+ from glomerular filtrate. Thus, gills, gastrointestinal tract, and kidneys all play crucial roles in water-salt and osmotic balance regulation in teleosts.

Na+ and Cl- balance is primarily achieved through the activity regulation of transporter proteins in gill, gastrointestinal, and renal tubular epithelia, including Na+/K+-ATPase (NKA), Na+-K+-2Cl- cotransporter (NKCC), Na+/H+ exchanger (NHE), and cystic fibrosis transmembrane conductance regulator (CFTR). This review examines the functions and regulatory mechanisms of these major transporters to provide theoretical support for research and practical ap-

plications in fish osmoregulation.

NKA is a P-type $(\alpha\beta)_2$ protein comprising four α subunits and three β subunits. As a transmembrane protein and the primary active pump providing driving force for ion transport in osmoregulatory tissues of teleosts, its main function is to transport three Na+ ions out of the cell while importing two K+ ions, thereby maintaining cellular homeostasis and energizing numerous transport systems. Most euryhaline teleosts adapt to varying environmental salinities by modulating NKA activity, with the enzyme establishing electrochemical gradients for Na+ and Cl- transport across the basolateral membrane of epithelial cells in both marine and freshwater conditions.

Studies have shown that gill NKA responses to environmental salinity correlate with fish ecological habits. In Mozambique tilapia (*Oreochromis mossambicus*), gill NKA activity is lowest in natural freshwater habitats and increases under hyperosmotic conditions. Conversely, in milkfish (*Chanos chanos*), gill NKA activity is lowest in natural seawater and increases under hyposmotic conditions. Research on medaka (*Oryzias latipes*) and brackish medaka (*Oryzias dancena*) revealed that when these species inhabit seawater and freshwater, respectively, gill NKA α subunit mRNA expression peaks, while NKA activity and α subunit protein abundance are lowest when they reside in their natural salinities. Similar patterns have been observed in chum salmon (*Oncorhynchus keta*), Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), killifish (*Fundulus heteroclitus*), striped bass (*Morone saxatilis*), and Nile tilapia (*Oreochromis niloticus*). These findings suggest that gill NKA activity may reflect the degree of environmental adaptation, being lowest when fish inhabit their natural salinity.

Euryhaline fish adapt to salinity changes by modulating gill NKA activity, which likely results from differential expression of its subunits. In rainbow trout, transfer from freshwater to 80% seawater decreased gill NKA $ $1a mRNA expression while increasing $ $1b expression, with $ $1c and $ $3 remaining unchanged. In medaka, intestinal NKA α expression remained constant at 5, 15, and 25 g/L NaCl, whereas NKA β expression was significantly inhibited at 15 and 25 g/L. These differential expression patterns indicate that NKA β functions primarily in hyposmotic environments, while NKA α operates in both hyposmotic and hyperosmotic conditions. The distinct expression of gill NKA $ $1a and $ $1b suggests they may regulate osmotic balance in low- and high-salinity environments, respectively, explaining how euryhaline fish adapt to broad salinity ranges.

Environmental salinity also affects NKA expression in intestine and kidney. In Mozambique tilapia, intestinal NKA α expression was significantly higher in seawater than freshwater. In Japanese eel (*Anguilla japonica*), gill NKA α expression was higher in seawater, while renal expression was lower compared to freshwater. These observations demonstrate that NKA in multiple osmoregulatory tissues contributes to osmotic balance, with intestinal NKA in some species showing greater sensitivity to salinity changes than gill NKA. In mullet (*Mugil cephalus*) juveniles, intestinal NKA activity exceeded gill activity at 20‰

salinity. In marine fish, intestinal NKA activity is generally high, sometimes surpassing gill NKA activity. The salinity-induced changes in NKA activity across multiple tissues underscore its importance in stabilizing body fluid osmolality. Furthermore, studies on Japanese seabass (*Lateolabrax japonicus*) revealed that long-term feeding with low-magnesium diets (0.413 g/kg) reduced gill NKA sensitivity to acute salinity stress compared to high-magnesium diets (1.042–1.991 g/kg), confirming that dietary mineral content significantly influences homeostasis. Therefore, both environmental and dietary mineral factors must be considered in fish osmoregulation research.

2 NKCC and NCC

As members of the solute carrier family 12A (SLC12A), NKCC proteins are membrane transporters located on the apical or basolateral membranes of epithelial cells, mediating ion absorption and secretion by simultaneously moving one Na+, one K+, and two Cl- ions into epithelial cells along their electrochemical gradients. Two NKCC isoforms have been identified in fish: basolateral NKCC1 (secreting ions to the external environment) and apical NKCC2 (absorbing ions into the body). In hyperosmotic environments, NKCC1 is activated to secrete ions and maintain cellular osmotic balance. Two NKCC1 subtypes (NKCC1a and NKCC1b) have been cloned from European eel (*Anguilla anguilla*) and Mozambique tilapia, with NKCC1a expressed in most tissues and NKCC1b predominantly in brain. NKCC2 is primarily expressed on the apical membrane of intestinal and renal tubular epithelial cells, mediating NaCl absorption from the intestinal lumen in marine teleosts.

Salinity changes affect NKCC gene expression in fish tissues. In killifish transferred from freshwater to seawater, gill NKCC1 mRNA expression increased. In *Sarotherodon melanotheron*, gill NKCC1a mRNA expression was significantly higher at 136‰ salinity than at 0‰. In three salmonid species, gill NKCC expression increased with environmental salinity, mirroring NKA activity changes. In European seabass (*Dicentrarchus labrax*), gill NKCC1 mRNA expression was lower in freshwater than seawater, while renal and posterior intestinal NKCC expression showed minimal salinity effects. In *S. melanotheron*, gill NKCC1a expression far exceeded that in intestine and kidney at 136‰ salinity, indicating gills as the primary site for ion secretion in marine environments. In European eel, transfer of yellow eels (pre-migratory) to seawater increased gill NKCC1a mRNA expression 4.3-fold within 2 hours and nearly 6-fold after 3 weeks, while expression decreased in kidney and mid-intestine. In contrast, silver eels (migratory) showed no significant gill NKCC1a changes, with renal expression lower than in seawater-acclimated yellow eels. These findings demonstrate that gill NKCC1a secretes excess salts in hyperosmotic environments, while intestinal and renal NKCC1a-mediated secretion is relatively limited, and that ecological habits influence NKCC1a expression patterns.

Hormones also modulate NKCC expression. Cortisol directly stimulates gill NKCC1 mRNA expression in brown trout and Atlantic salmon, while pituitary

prolactin regulates intestinal NKCC2 expression to adapt to salinity changes in Mozambique tilapia.

Another SLC12A family member, Na+/Cl- cotransporter (NCC), is present on the apical membranes of ionocytes and yolk sac membranes in some freshwater fish. This electroneutral transporter simultaneously facilitates Na+ and Cl- absorption, with low activity during seawater adaptation. In Japanese seabass, NCC mRNA expression in gill, intestine, and kidney was significantly higher in freshwater than seawater, peaking on day 3 post-transfer. In Mozambique tilapia, gill NCC mRNA expression increased in freshwater, particularly under "normal Na+/low Cl-" conditions, indicating its primary role in Cl- absorption. Basolateral NKCC1a is expressed in type IV mitochondria-rich cells in seawater, mediating ion secretion, whereas apical NCC is expressed in type II mitochondria-rich cells in freshwater, mediating ion absorption. Thus, marine fish primarily utilize basolateral NKCC1 for ion secretion, while freshwater fish employ apical NKCC2 and NCC for ion absorption. Although numerous studies have examined salinity effects on NKCC and NCC activity and expression, the influence of dietary minerals requires further investigation.

NHE is a bidirectional ion exchanger located on apical or basolateral membranes that transports Na+ into cells in exchange for H+ (or NH4+), mediating electroneutral 1:1 Na+/H+ exchange. Since Krogh proposed the coupling of Na+ absorption and acid secretion, this exchange mechanism has been recognized as a potential pathway for Na+ uptake. In zebrafish, eight NHE isoforms have been identified, with NHE2 and NHE3b highly expressed in gills, NHE3a and NHE3b in kidney, and NHE8 ubiquitously expressed. Inhibition studies revealed that NHE and H+-ATPase play crucial roles in Na+ absorption in mitochondria-rich cells, with the transcription factor foxi3a regulating their differentiation. NHE mRNA expression is sensitive to environmental salinity, pH, and hormones. In stingrays (*Dasyatis sabina*), low salinity increased gill NHE3 expression to enhance Na+ uptake. In Mozambique tilapia, gill NHE3 mRNA expression was higher in freshwater than seawater. In rainbow trout transferred to 65% seawater, intestinal NHE3 expression increased to secrete H+ and neutralize HCO3- secreted by epithelial cells, maintaining intestinal acid-base balance. Elevated plasma cortisol concentrations, induced by high environmental CO2, significantly increased renal NHE mRNA and protein abundance. In acid-tolerant dace (*Tribolodon hakonensis*), gill NHE3 mRNA expression increased dramatically upon acid exposure, promoting H+ excretion and Na+ absorption. These results demonstrate that NHE participates in both osmotic and acid-base regulation, with its activity modulated by hormones, salinity, and pH through multiple pathways. Differential expression of NHE isoforms under identical conditions suggests functional specialization. In killifish transferred from 10% seawater to freshwater, gill NHE2 expression exceeded NHE3 after 12 hours, while in rainbow trout kidney, NHE3 expression surpassed NHE2. Thus, NHE functions differ by environment: in seawater, NHE primarily maintains acid-base balance, whereas in freshwater, it mainly regulates osmotic pressure. When investigating dietary mineral effects on osmoregulation via NHE, environmental salinity must

be considered, and differential isoform expression should be accounted for.

CFTR is an anion channel regulating Cl- transport, located in mitochondria-rich chloride cells and opercular epithelial cells of teleost gills, activated by cAMP and protein kinase A. In killifish transferred from freshwater to seawater, gill, opercular epithelial, and intestinal CFTR expression increased significantly. Conversely, transfer from seawater to freshwater reduced CFTR expression within 24 hours, a pattern also observed in tilapia. High CFTR expression in seawater and low expression in freshwater indicates its critical role in Cl- secretion by mitochondria-rich cells to maintain Cl- balance in euryhaline teleosts. Two CFTR isoforms exist in Atlantic salmon: CFTR expression increased significantly within two weeks of seawater transfer, while CFTR showed only transient upregulation during the initial 24 hours, suggesting CFTR primarily responds to acute salinity changes. Therefore, studies on CFTR-mediated Cl- metabolism must consider both activity and the timing of sampling, as well as isoform-specific expression patterns.

In summary, NKA, NKCC, NCC, NHE, and CFTR all participate in osmoregulation in euryhaline teleosts. Basolateral NKA hydrolyzes ATP to transport Na+ out of cells, creating Na+ gradients that drive NHE, NKCC, and NCC-mediated Na+ and Cl- transport. CFTR in gill chloride cells and opercular epithelia primarily regulates Cl- metabolism, mediating Cl- secretion in hyperosmotic environments to maintain osmotic homeostasis. While most research has focused on salinity effects on these transporters, few studies have examined dietary mineral influences. This review provides a theoretical foundation for investigating mechanisms by which dietary minerals regulate fish osmotic balance.

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